

CLAIM LISTING

This listing of claims replaces all prior versions and listings of claims in the application.

1-16 (canceled)

17. (previously presented) A hybridization assay for at least one of a multiplicity of nucleic acid sequences in an analyte comprising the steps of:

- (a) contacting said analyte with a mixture of encoded microcarriers having immobilized on their surfaces
 - (i) a hybridization probe for one of said multiplicity of sequences, whose hybridization to said at least one sequence can be detected, and
 - (ii) a coding scheme comprising a plurality of signaling hairpins that are not hybridization probes for said multiplicity of sequences, including said at least one sequence, comprising quenched, fluorophore-labeled hairpin molecules each comprising an interacting affinity pair separated by a linking moiety, one member of said affinity pair having bound thereto at least one quenched fluorophore, wherein interaction of the affinity pair is disruptable to unquench said at least one fluorophore by a physical or chemical change in a condition of its environment, wherein the disruption of the interaction of at least one affinity pair occurs at a first level of said condition and the disruption of the interaction of at least another affinity pair occurs at a second level of said condition, and wherein said disruptions are optically differentiable, and wherein the coding scheme for identifying individual microcarriers in said mixture comprises a combination of multiple spectrally differentiable fluorophores and multiple affinity pairs disruptable at detectably different levels of said condition;
- (b) forming a distributed array of said microcarriers wherein location of said microcarriers in said distributed array is not used to identify said at least one nucleic acid sequence;
- (c) determining which microcarriers have hybridization probes hybridized to said at least one nucleic acid sequence of said analyte; and
- (d) optically decoding the microcarriers having said at least one nucleic acid sequence hybridized to its hybridization probes to identify said at least one nucleic acid sequence

by changing said condition to said detectably different levels to disrupt quenching, and detecting changes in fluorescence from the signaling hairpins.

18. (previously presented) The assay according to claim 17, wherein said interacting affinity pair comprises complementary oligonucleotide sequences hybridized to one another.

19. (previously presented) The assay according to claim 18 wherein said mixture of signaling hairpins includes at least three affinity pairs.

20. (previously presented) The assay according to claim 18 wherein said mixture of signaling hairpins includes from three to eight affinity pairs.

21. (previously presented) The assay according to claim 19 wherein steps (c) and (d) include decoding all microcarriers.

22. (previously presented) The assay according to claim 18, wherein said linking moiety comprises an oligonucleotide sequence.

23. (previously presented) The assay according to claim 18, wherein the step of decoding includes disrupting the hybridized affinity pairs by increasing temperature.

24. (previously presented) The assay according to claim 22, wherein forming the distributed array comprises immobilizing individual microcarriers at the ends of fibers in a fiber-optic bundle.

25. (previously presented) The assay according to claim 18, wherein steps (c) and (d) include flow cytometry.

26. (previously presented) The assay according to claim 18, wherein a quencher is attached to the complementary oligonucleotide sequence not bearing the at least one fluorophore.

27. (previously presented) The assay according to claim 18, wherein step (a) precedes step (b).

28. (previously presented) The assay according to claim 18, wherein step (b) precedes step (a).

29. (previously presented) The assay according to claim 28, wherein said distributed array is a planar array.

30. (previously presented) The assay according to claim 17, wherein the step of forming a distributed array comprises immobilizing individual microcarriers at the ends of fibers in fiber-optic bundles.

31. (previously presented) The assay according to claim 17, wherein step (a) precedes step (b).

32. (previously presented) The assay according to claim 17, wherein step (d) includes disrupting said affinity pairs by increasing temperature.

33. (previously presented) The assay according to claim 17, wherein step (d) includes disrupting said affinity pairs by adding a denaturant.

34. (previously presented) The assay according to claim 17, wherein steps (c) and (d) include flow cytometry.

35. (previously presented) The assay according to claim 17, wherein said distributed array is a planar array.

36. (previously presented) The assay according to claim 17, wherein said distributed array is a linear array.

37. (previously presented) The assay according to claim 17, wherein said hybridization_probe is a molecular beacon probe.

38. (previously presented) The assay according to claim 17, wherein step (c) includes determining how much of said at least one nucleic acid sequence has hybridized.